PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12176752/EJH/MLO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/AU2003/000696	International Filing Date *(day/month/year)* 4 June 2003	Priority Date *(day/month/year)* 4 June 2002
International Patent Classification (IPC) or national classification and IPC Int. Cl.[7] C12Q 1/68		
Applicant THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheet(s).

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

   IV [ ] Lack of unity of invention

   V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [ ] Certain defects in the international application

   VIII [ ] Certain observations on the international application

Date of submission of the demand 8 December 2003	Date of completion of the report 29 March 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer TERRY MOORE Telephone No. (02) 6283 2632

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/000696

I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1-30,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **31-34** , received on **18 March 2004** with the letter of **17 March 2004**

☒ the drawings, pages **1-10,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the sequence listing part of the description:
pages **1 and 2,** as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language,** all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-15	YES
	Claims	NO
Inventive step (IS)	Claims 1-15	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-15	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

The specification describes an anchoring system for nucleic acid molecules comprising a solid phase support covalently linked to a tag oligonucleotide, which is in turn used as a substrate for ligase-mediated covalent bonding to a target nucleic acid molecule. Ligation is mediated by association of the tag oligonucleotide with a complementary bridging oligonucleotide that, when hybridised to the tag oligonucleotide leaves a 5' overhang in the bridging oligonucleotide to which the target nucleic acid can hybridise. The target nucleic acid can then be ligated to the tag oligonucleotide. The system is particularly useful for transcription, translation and amplification reactions.

The following documents identified in the International Search Report have been considered for the purposes of this report:

D1 WO 2001 48244
D2 US 6 030 782
D3 Rogers et al (1999) Analytical Biochemistry 266, 23-30
D4 Zhang et al (1991) Nucleic Acids Research 9, 3929-33

Novelty and Inventive Step

D1 discloses a system comprising a solid phase support covalently linked to a first oligonucleotide where both the first oligonucleotide and a second nucleic acid sequence are complementary to a third nucleic acid sequence. When both the first and second nucleic acids hybridise with the third sequence the first and second nucleic acids are adjacent to one another and can be ligated to covalently link the first and second nucleic acids (see page 14). However D1 does not disclose an α-tag that hybridises to the first nucleic acid leaving a 3' overhang in the first nucleic acid.

D2-D4 all disclose nucleic acids covalently linked to solid supports. D3 and D4 specifically disclose linkage to membranes and glass slides whereas D2 discloses linkage to a range of solid supports, including microspheres, membranes and beads. The citations also disclose a range of linkage methods, including those specifically claimed in claims 4 and 7. However none of D2-D4 disclose combinations of a solid phase covalently linked to a tag oligonucleotide where the tag is hybridised to an α-tag, leaving a 3' overhang on the tag that is used as substrate for ligase-mediated covalent bonding to a target nucleic acid.

As such none of the citations clearly disclose or teach toward systems comprising all of the features of the claims.

CLAIMS

1. A solid phase comprising a surface first chemical moiety which participates in covalent bond formation with a second chemical moiety conjugated to a tag oligonucleotide rendered partially double stranded by annealing an α-tag oligonucleotide to the tag oligonucleotide to provide a 3' overhang portion of the tag oligonucleotide wherein the tag oligonucleotide is employed as a substrate for ligase-mediated covalent bonding to a target nucleic acid molecule.

2. The solid phase of Claim 1 comprising a solid support in the form of a microsphere, microchip or a glass, plastic or ceramic slide.

3. The solid phase of Claim 2 wherein the solid support is a microsphere.

4. The solid phase of Claim 1 wherein the surface chemical moiety is capable of covalent bond formation with an amine group, a thiol group or an acryl group.

5. The solid phase of Claim 1 wherein the surface chemical moiety is capable of covalent bond formation with a carboxyl group.

6. The solid phase of Claim 1 wherein the surface chemical moiety is a carboxyl group.

7. The solid phase of Claim 1 wherein the second chemical moiety is an amine group.

8. The solid phase of Claim 1 wherein the tag oligonucleotide comprises a chemical moiety conjugated to a known oligonucleotide sequence *via* a molecule comprising mC+n atoms, from about 1 to about 100, wherein m is the number of repeats of size C and n is the number of atoms not included in the repeats.

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2. The solid phase of Claim 1 comprising a solid support in the form of a microsphere, microchip or a glass, plastic or ceramic slide.

3. The solid phase of Claim 2 wherein the solid support is a microsphere.

4. The solid phase of Claim 1 wherein the surface chemical moiety is capable of covalent bond formation with an amine group, a thiol group or an acryl group.

5. The solid phase of Claim 1 wherein the surface chemical moiety is capable of covalent bond formation with a carboxyl group.

6. The solid phase of Claim 1 wherein the surface chemical moiety is a carboxyl group.

7. The solid phase of Claim 1 wherein the second chemical moiety is an amine group.

8. The solid phase of Claim 1 wherein the tag oligonucleotide comprises a chemical moiety conjugated to a known oligonucleotide sequence *via* a molecule comprising mc+n atoms, from about 1 to about 100, wherein m is the number of repeats of size C and n is the number of atoms not included in the repeats.

9. The solid phase of Claim 1 wherein the α-tag oligonucleotide is labeled with a reporter molecule and is phosphorylated at its 5' end.

10. The solid phase of Claim 8 or 9 further comprising a bridging oligonucleotide, said bridging oligonucleotide having a nucleotide sequence complementary to the nucleotide sequence of the 3' overhang portion of the tag oligonucleotide and a nucleotide sequence complementary to a terminal end portion of a target nucleic acid molecule.

11. The solid phase of Claim 10 further comprising a target nucleic acid molecule in ligase-mediated covalent bonding to the tag oligonucleotide molecule anchored to the solid phase.

12. A substrate for anchoring a target nucleic acid molecule, said substrate comprising:-

(i) a solid phase having a first chemical moiety on its surface;

(ii) a tag oligonucleotide comprising a second chemical moiety in covalent bond formation with the first chemical moiety, said second chemical moiety conjugated to the tag oligonucleotide *via* a molecule of structure mc+n from about 1 to about 100, wherein m is the number of repeats of size c and n is the number of atoms not included in the repeats.;

(iii) an optionally labeled α-tag oligonucleotide complementary to the tag oligonucleotide resulting in a 3' singled-stranded overhang of the tag oligonucleotide; and

(iv) a bridge oligonucleotide having complementary based to the 3' overhang region of the tag oligonucleotide and complementary bases to the 5' end portion of the target nucleic acid molecule wherein the target nucleic acid molecule is anchored to the tag oligonucleotide *via* ligase-mediated conjugation.

14. A universal nucleic acid anchoring system comprising the structure:-

$$S(-T)_p$$

wherein:

S is a solid support having a chemical moiety capable of covalent bond formation with a second chemical moiety;

T is a partially double-stranded oligonucleotide comprising a single-stranded tag oligonucleotide having said second chemical moiety linked *via* a spacer molecule to its 5' end, said spacer comprising carbon atoms having the structure mc+n from about 1 to about 100, wherein m is the number of repeats of size c and n is the number of atoms not included in the repeats, said tag oligonucleotide further comprising a complementary oligonucleotide (α-tag) annealed to the tag oligonucleotide to provide a 3' overhang or sticky end, single-stranded nucleotide sequence, on the tag oligonucleotide; said T further comprising a bridging oligonucleotide having a nucleotide sequence complementary to the 3' overhang nucleotide sequence on the tag oligonucleotide and a further nucleotide sequence complementary to a nucleotide sequence on the 5' end of a target nucleic acid molecule;

wherein T may be represented p times on the solid support wherein p is from about 1 to about 100,000.

15. A method for immobilizing a target nucleic acid molecule to a partially double-stranded tag oligonucleotide anchored to a solid support, said method comprising ligating a phosphorylated 5' end of the target nucleic acid molecule to a complementary single-stranded portion of the tag oligonucleotide under conditions to permit ligase-mediated covalent bond formation wherein said tag oligonucleotide is covalently anchored to the solid support *via* covalent bond formation between a first chemical moiety on the surface

of the solid support and a chemical moiety conjugated to the tag oligonucleotide *via* a molecule of structure mc+n from about 1 to about 100, wherein m is the number of repeats of size c and n is the number of atoms not included in the repeats wherein the tag oligonucleotide is rendered partially double-stranded by annealing a complementary oligonucleotide to the tag oligonucleotide leaving a single-stranded 3' terminal portion of the tag oligonucleotide which is used to capture the target nucleic acid molecule *via* a bridging oligonucleotide.